The Y Chromosome Effect on Blood Pressure in Two European Populations


Abstract—Higher blood pressure (BP) in males compared with females is well documented and is thought to be influenced in part by the Y chromosome. To examine whether there is an association between BP and a polymorphic HindIII biallelic marker in the nonrecombining region of the Y chromosome, we genotyped 155 males from a Polish study group and 762 males from a Scottish study group. We also tested for possible interaction between the Y chromosome and a mutation in the steroidogenic factor binding site of the aldosterone synthase gene by genotyping the same group from Scotland. There was no significant difference in age or body mass index between 2 Y chromosome genotypes in both study groups. Men with the HindIII(+) genotype had significantly higher systolic and diastolic pressures than those with the HindIII(−) genotype in both the Polish and Scottish studies. This difference between the genotypes was 5.27 mm Hg (P=0.0014) and 3.14 mm Hg (P=0.0005) for adjusted systolic BP and 2.6 mm Hg (P=0.0045) and 1.44 mm Hg (P=0.0084) for adjusted diastolic BP in the Polish and the Scottish studied, respectively. On binary logistic regression analysis, males with the HindIII(+)/TT SF1 genotype combination had an odds ratio for elevated BP of 3.92 (CI 1.21 to 12.68, P=0.023). Our results indicate that the Y chromosome harbors a locus or loci that contribute to BP variation in hypertensive and normotensive men. The polymorphism in the aldosterone synthase gene may interact with the Y chromosome to increase the odds of an individual’s developing higher BP. (Hypertension. 2002;39[part 2]:353-356.)

Key Words: hypertension, essential, genetics, gender, genes, blood pressure, aldosterone

Men have a higher prevalence of hypertension and ischemic heart disease compared with women; this is contributed, in part, by the sex difference in blood pressure (BP) that occurs throughout their life span.1 One of the obvious genetic determinants of sexual dimorphism is the Y chromosome. The Y exists as a single chromosome, and the majority of this chromosome does not recombine (known as the nonrecombining region [NRY]) and is inherited intact by sons from their fathers.2 Extensive data from rodent models indicate a significant association between the Y chromosome and BP.3–8 Moreover, human studies have shown that a hypertensive father but not a mother affects BP in normotensive male offspring.9 Recently, Ellis et al10 reported an association between an increased risk of elevated diastolic blood pressure (DBP) and a biallelic polymorphism on the NRY region of the Y chromosome in 409 subjects recruited from the general population in Victoria, Australia. However, it cannot be excluded that other polymorphic loci contained within the NRY region of the Y chromosome or other autosomal loci might interact with the HindIII(+/−) variant to affect systolic BP (SBP) and DBP. Among such candidate loci, genes coding for components of aldosterone system seem to be of major importance considering their pivotal role in BP regulation.11 Furthermore, sexual dimorphism in aldosterone secretion has been reported in experimental models of essential hypertension.12 In addition, aldosterone synthase gene (CYP11B2) has been shown to interact with other autosomal genes to affect BP.13 In light of these data, the CYP11B2 gene seems to be an outstanding candidate that might interact with the Y chromosome to affect BP in males.

In the present study, we tested the hypothesis that the Y chromosome polymorphism was associated with elevated BP in 2 European populations. We also tested for interactions between the Y chromosome and a polymorphism in the steroidogenic factor (SF1) binding site of CYP11B2 gene.

Methods

Subjects
The Silesian Hypertension Study was designed to investigate the genetic predisposition to several cardiovascular phenotypes and was

Received September 25, 2001; first decision November 7, 2001; revision accepted November 21, 2001.
From the British Heart Foundation Blood Pressure Group (F.J.C., M.T., S.P., M.N.U., G.C.I., N.H.A., A.M., J.M.C.C., G.C.M.W., A.F.D.), Department of Medicine and Therapeutics and Department of General Practice, University of Glasgow, United Kingdom; and Department of Internal Medicine, Diabetology and Nephrology (B.L., E.Z.-S., W.G.), Silesian School of Medicine, Zabrze, Poland.
Correspondence to Professor Anna F. Dominiczak, Department of Medicine and Therapeutics, University of Glasgow, Western Infirmary, Glasgow G11 6NT, United Kingdom. E-mail ad7e@clinmed.gla.ac.uk.
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based on collecting hypertensive probands and their families. A total of 629 white individuals representing 212 pedigrees were recruited from the south of Poland. Of 313 males in this cohort, 155 unrelated men were selected for additional Y chromosome analysis. Each of the 155 selected men represented parental or offspring generation from 1 family. This selection was performed to avoid potential bias that might be produced by an excess of identical Y chromosomes from families with more than 1 male.

Basal phenotyping included taking clinical history according to the recommendations of the World Health Organization Guidelines for the Management of Hypertension and was followed by physical examination, basic laboratory tests, and completion of standardized questionnaires. Height and weight measurements were taken in standard conditions, and body mass index (BMI) was calculated using the equation BMI=W/H² (where W is weight in kilograms and H is height in meters squared). BP was taken in a sitting position using a mercury sphygmomanometer with a cuff size individually adjusted to the arm after 20 minutes of rest. SBP was taken at the return of arterial sounds (Korotkoff phase 1), and disappearance of sounds (Korotkoff phase V) indicated DBP. The average of 3 readings was taken and the mean of the last 2 was used. MAP was adjusted to the arm after 20 minutes of rest. 3 readings were taken and the mean of the last 2 was used. There was no calibration drift during the study, based on daily static calibration checks against a mercury test gauge. MAP and BMI were calculated as described above. Blood samples were taken from each individual for biochemical analyses and DNA isolation. The study was approved by the local Bioethical Committee, and informed consent was obtained from each individual who participated in the study.

The Scottish study was based on a cross-sectional study of 1040 men who were aged 30 to 59 and composed the second generation of the MIDSPAN family study described previously.14,15 A total of 762 unrelated men from the offspring population were chosen for the current study. BP measurements were obtained using an automated Dinamap 8100 instrument with the correctly sized cuff applied to the left arm while the subject was in the sitting position. After 5 minutes of rest, 3 readings were taken and the mean of the last 2 was used. There was no calibration drift during the study, based on daily static calibration checks against a mercury test gauge. MAP and BMI were calculated as described above. Blood samples were taken from each individual for biochemical analyses and DNA isolation. The study was approved by the Argyll and Clyde Local Research Ethics Committee, and informed consent was obtained from all participants.

**Molecular Analysis**

**Y Chromosome Restriction Fragment Length Polymorphism**

Genotype analysis for the Y chromosome restriction fragment length polymorphism (RFLP) was performed using approximately 50 ng of DNA from each participant. This RFLP was detected by amplification in a single polymerase chain reaction (PCR); the following sense and antisense primers were used: 5'-TCTGAGACATCTCTTGTGGA-3' and 5'-CGCTCAAATATCCACCTTTCAG-3'; DNA was added to a mix that contained 10 pmol of each primer, 1× polymerase chain reaction buffer (Qiagen), 10 μM each of the 4 dNTPs (Promega), 1.5 mM MgCl₂, and 1 U Hot-start Taq DNA polymerase (Qiagen) to give a total reaction volume of 20 μL. The first denaturation step at 95°C for 10 minutes (for activation of the Taq DNA polymerase) was followed by 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds, followed by a final extension time at 72°C for 10 minutes. The DNA products were then digested by the addition of 5 U of HindIII restriction endonuclease (Promega) in the presence of 1× buffer (Promega) at 37°C for 12 hours. Digested products were resolved on a 2% 1000-agarose (Life Technologies) gel that contained ethidium bromide and visualized with Fluoro-S Multi-imager (Biorad). The digestion after amplification yields 2 possible genotypes of the biallelic markers located near the centromere region: A 290-bp uncut product was termed HindIII (−) or 3 products of 290, 250, and 40 bp, which were termed HindIII (+).

**Statistical Analysis**

Data are presented as mean and SD unless stated otherwise. Differences between genotype groups were compared by Welch’s corrected t test. A value of P<0.05 on a 2-sided test was considered significant. In some analyses, BP was adjusted for age and BMI by including these phenotypes as covariates in ANOVA. To test for the effect of both the Y genotype and SF1 genotype on BP, we used binary logistic regression analysis. Statistical analysis was performed using MINITAB statistical software version 13 for Windows.

**Results**

The general characteristics of the participants from the Silesian Hypertensive Study and MIDSPAN study are presented in Table 1.

The HindIII (−) genotype was found in 67% of the men in the Silesian Hypertensive Study and in 72% of the men in the MIDSPAN study. There was no significant difference in age or BMI between the Y chromosome genotypes in both study groups. Men with the HindIII (+) genotype had significantly higher SBPs than those with the HindIII (−) genotype in the Polish study (Table 2). This difference was 5.27 mm Hg (t value=3.2, P=0.0014) for SBP adjusted for age and BMI. The difference in SBP was also found in the MIDSPAN population (Table 2) and was 3.14 mm Hg (t value=5.86, P=0.0005) for values adjusted for age and BMI. Men with the HindIII (+) genotype also had significantly (P<0.05) higher DBP than those with the HindIII (−) genotype in the Polish study (Table 2). This difference was 2.6 mm Hg (t value=2.88, P=0.0045) for BPs adjusted for BMI and age. The difference was smaller but significant in the MIDSPAN subjects (Table 2). The mean difference in DBP adjusted for age and BMI was 1.44 mm Hg (t value=2.64, P=0.0084). MAP was also significantly higher in the HindIII (+) genotype group in both studies (Table 2).

**Table 1. Characteristics of the Men Used in the Silesian Hypertension and MIDSPAN Y Studies**

<table>
<thead>
<tr>
<th></th>
<th>Silesian</th>
<th>MIDSPAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>155</td>
<td>762</td>
</tr>
<tr>
<td>Age (y)</td>
<td>53.9±10.7</td>
<td>44.9±6.3</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>138.6±20.1</td>
<td>130.7±15.3</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>86.9±11.8</td>
<td>79.1±10.1</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>104.2±13.6</td>
<td>96.3±11.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.4±6.1</td>
<td>174.8±6.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.6±14.0</td>
<td>80.7±13.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6±4.2</td>
<td>26.4±4.0</td>
</tr>
<tr>
<td>Antihypertensive treatment (n)</td>
<td>48</td>
<td>38</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>18</td>
<td>180</td>
</tr>
<tr>
<td>Diabetic history (n)</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Stroke history (n)</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are given in mean±SD.

**SF1 RFLP**

Genotype analysis for the SF1 RFLP was performed in the MIDSPAN population using approximately 50 ng of DNA from each participant as previously described.11 The individuals who genotyped the subjects had no clinical data available to them during assignment of genotypes for both polymorphisms.

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A total of 48 men were receiving antihypertensive treatment in the Silesian Hypertensive Study. This number was not significantly different between the 2 different genotypes. Only 38 men were on antihypertensive therapy in the MIDSPAN study. The inclusion or exclusion of these individuals did not alter the results of the analysis.

There was no significant association of the SF1 genotype and BP in this study (data not shown, only in MIDSPAN population). In a logistic regression analysis, for SBP over 90 mm Hg, the odds ratio for the main effect of HindIII(+) relative to the HindIII(−) (with SF1 genotype maintained at CC) was 0.69 (CI 0.28 to 1.72, P=0.429) and those for the CT and TT SF1 genotype main effects (with HindIII(−)) were 0.75 (CI 0.41 to 1.36, P=0.347) and 0.68 (CI 0.36 to 1.31, P=0.249), respectively. However, compared with the HindIII(−)/SF1 CC genotype combination, the odds ratios were 4.25 (CI 1.49 to 12.15, P=0.007) for subjects who had both HindIII(+) and SF1 (CT), and 3.92 (CI 1.21 to 12.68, P=0.023) for subjects who had both HindIII(+) and SF1 (TT). Using the same parametric analysis, a logistic regression model for DBP over 90 mm Hg estimated the odds ratio for the effect of HindIII(+) relative to HindIII(−) as 0.63 (CI 0.18 to 2.18, P=0.470) and that for SF1 (CT) and SF1 (TT) as 1.50 (CI 0.72 to 3.12, P=0.283) and 1.40 (CI 0.64 to 3.04, P=0.400), respectively. Compared with the HindIII(−)/SF1 CC genotype combination, the odds ratio were 2.36 (CI 0.60 to 9.24, P=0.219) for subjects who had both HindIII(+) and SF1 (CT), and 4.15 (CI 0.97 to 17.75, P=0.055) for subjects who have both HindIII(+) and SF1 (TT) for DBP over 90 mm Hg. Both the above were corrected for BMI and age.

Unadjusted characteristics of individuals according to the Y chromosome HindIII and SF1 genotype combination in the MIDSPAN study are shown in Table 3.

### Discussion

Findings from this study of the 2 groups of men from Poland and Scotland showed that there was a significant association between a variant of the Y chromosome and an increase of SBP and DBP. This is additional evidence that the Y chromosome contributes to increased BP. The substantial differences in BP seen in this study could have a significant contribution to the cardiovascular morbidity and mortality rates of men.17

We found that the HindIII(+) genotype of the biallelic marker was significantly associated with higher SBP and DBP. This indicates that the marker on the Y chromosome is linked to a gene that affects BP. The relationship between HindIII(+/−) polymorphism and BP was previously reported in the Australian population by Ellis et al.10 Their results showed higher DBP associated with the HindIII(−) allele. This might be due to various reasons, the most obvious being the different demographics of the European and Australian populations. The Australian population studied is largely derived from families of heterogeneous European descent, and it is not known whether these will have a common ancestral background. Our study used populations derived from 2 distinct European areas of limited migration where the probability would be that the Y chromosome between indi-

### TABLE 2. Unadjusted Characteristics of Individuals According to the Absence [HindIII(−)] or presence [HindIII(+) of the Y Chromosome HindIII Restriction Site in the Silesian Hypertension and MIDSPAN Y Studies

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Silesian</th>
<th>MIDSPAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HindIII(−)</td>
<td>HindIII(+)</td>
</tr>
<tr>
<td>n</td>
<td>104</td>
<td>51</td>
</tr>
<tr>
<td>Age (y)</td>
<td>52.8±10.9</td>
<td>54.8±10.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9±4.1</td>
<td>27.4±4.6</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>135.3±16.1</td>
<td>145.3±25.3</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.3±10.1</td>
<td>90.4±14.0</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>102.0±11.2</td>
<td>108.7±16.8</td>
</tr>
</tbody>
</table>

Values are given in mean±SD. CC, CT, and TT represent the 3 SF1 genotypes.

### TABLE 3. Unadjusted Characteristics of Individuals According to the Y Chromosome HindIII and SF1 Genotype Combination in the MIDSPAN Study

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>HindIII(−)</th>
<th>HindIII(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>n</td>
<td>101</td>
<td>267</td>
</tr>
<tr>
<td>Age (y)</td>
<td>44.5±6.3</td>
<td>44.8±6.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1±3.5</td>
<td>26.3±4.0</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>130.2±13.5</td>
<td>128.4±13.6</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>79.3±10.3</td>
<td>78.8±10.9</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>96.2±10.3</td>
<td>95.0±11.0</td>
</tr>
</tbody>
</table>

Values are given as mean±SD. CC, CT, and TT represent the 3 SF1 genotypes. P values obtained with ANOVA. Because of multiple testing, significance level of 0.005 is appropriate for the above table.
viduals is related by ancestry. In fact, one cannot discount founder effect in this case. A high level of differentiation is documented within European populations, and the demographic history of Europe is thought to be complex and influenced by major population movements, linguistic and geographic barriers, and genetic drift. We cannot postulate whether the populations studied will have a similar ancestry or whether a recombination event during evolution has caused this change. Haplotype analysis will lead to a better understanding of this question. Another factor that can confound our results is a gene or genes that contribute to BP on the pseudoautosomal regions of the Y chromosome. These regions undergo recombination with the X chromosome during meiosis.

This study is the first to report interaction between the Y chromosome and an autosomal gene. This interaction is not surprising in light of the polygenic nature of BP regulation. Furthermore, in several experimental models of essential hypertension, there are strong links between the Y chromosome and other physiological parameters that can affect BP, such as androgens, sympathetic nervous system, and salt sensitivity. The CYP11B2 SF1 T allele has been previously found to be associated with hypertension. Mutations in the aldosterone synthase gene are also known to result in glucocorticoid remediable hyperaldosteronism, which is an autosomal dominant form of hypertension. Here we report that a polymorphism in the CYP11B2 gene SF1 binding site may interact with the Y chromosome to increase the odds of an individual’s developing high BP. The nature of this interaction is unclear as the SF1 polymorphism in CYP11B2 does not alter expression of aldosterone synthase. Nevertheless, the T allele is associated with increased urinary aldosterone excretion. The reason for this remains to be determined, but it is likely that the polymorphism is in linkage disequilibrium with other functional variants at this locus.

The question that remains to be answered is what gene or genes on the Y chromosome actually contribute to BP regulation? This gene may have an important general physiological function because the Y chromosome is thought to have evolved from a full-sized autosome, and a gene that leads to higher mortality in males might be too deleterious to be conserved. The human Y sequence was published recently, and this will allow us to develop single nucleotide polymorphisms to perform haplotype analysis. Combined linkage disequilibrium and functional studies in humans and in rat consomic strains will allow additional dissection of the gene or genes responsible for BP regulation.

Acknowledgments
The study was supported by a British Heart Foundation Programme grant and EURNETGEN EC Programme grant to A.F.D., Wellcome Trust Fellowships to J.C. and M.N.U., Chief Scientist Biomedical and Therapeutic research grant to J.M.C.C., MRC Cooperative Group grant, and NHS Cardiovascular R&D Programme grant to G.C.M.W.

References
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Hypertension. 2002;39:353-356
doi: 10.1161/hy0202.103413

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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